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Some initial animal and human pharmacological studies with benapryzine (BRL 1288)

D. M. BROWN, B. O. HUGHES, C. D. MARSDEN,* J. C. MEADOWS† AND B. SPICER

Beecham Research Laboratories, Brockham Park, Betchworth, Surrey RH3 7AJ

Summary

1. The pA2 anti-acetylcholine activity *in vitro* for benapryzine was 6·55 compared with 9·02 for benzhexol.

2. *In vivo*, the anti-acetylcholine activity of benapryzine relative to benzhexol was 0·038 as assessed by the mydriatic response of mice after subcutaneous administration. The relative activity assessed by the inhibition of pilocarpine-induced salivation was 0·13 after oral administration and 0·056 following subcutaneous administration of the drugs.

3. Benapryzine had the same order of activity as benzhexol in inhibiting oxotremorine-induced tremors in mice.

4. Benapryzine had anticonvulsant properties but no analgesic activity, whilst in high doses it antagonized the extrapyramidal symptoms induced by perphenazine in rats.

5. In patients benapryzine was effective in reducing the symptoms of Parkinson's disease without overt anti-cholinergic effects or central hallucinogenic actions.

6. Benapryzine abolished the excess tremor and reduced the rigidity and akinesia induced by physostigmine in Parkinsonian subjects.

Introduction

From a series of dialkylaminoethanol esters of diphenylacetic acid, it was found that the *N*-propylethyl ester (benapryzine) had significant central anticholinergic activity, with negligible peripheral effects. The pharmacology and clinical pharmacology of benapryzine are described in this paper. Drugs with central anticholinergic activity are used to treat patients with Parkinson's disease (see Duvoisin, 1967, for a review of the role of anticholinergic drugs in Parkinson's disease), so a preliminary evaluation of benapryzine in Parkinson's disease is also reported.

Methods

Studies in animals

Antiacetylcholine activity in vitro

A 2 cm section, from the terminal ileum of a guinea-pig weighing approximately 250 g was suspended in Tyrode solution at 30° C in a 5 ml gut bath. Regular

* University Department of Neurology (Institute of Psychiatry and King's College Hospital), De Crespigny Park, Denmark Hill, London, S.E.5.
† National Hospital for Nervous Diseases, Queen Square, London, W.C.1.

contractions were obtained to 5–30 ng acetylcholine with a 2 min contact period; a 3 min cycle time was used throughout the experiment. The pA2 was determined at 2 min according to the method described by Schild (1947).

In vivo *mydriatic activity*

Compounds were administered subcutaneously to groups of five male or female mice (18–22 g) which were kept under subdued lighting conditions. The pupil diameters were measured at 15, 20, 40, 60, 80 and 100 min after administration of drugs; measurement was on an arbitrary scale with a binocular dissecting microscope which had a focal light source directed at the eye. The results were plotted on arithmetic paper.

Antioxotremorine activity

The assessment of the inhibition of tremor was carried out by the method of Everett, Blockhus & Shepperd (1956) as described for tremorine, but modified as follows when oxotremorine was the tremorogenic agent. Groups of 10 female mice (18–22 g) were injected i.p. with 2 mg/kg of oxotremorine, in 0·2 ml saline (0·9% w/v NaCl solution) per 20 g mouse. The degree of tremor which developed was assessed 10 min after administration of the oxotremorine by a scoring method: 2 for full tremor, 1 for slight tremor and 0 for no tremor. The compounds under test were administered 10 min before oxotremorine for the subcutaneous test and 30 min before oxotremorine in the oral study. The degree of tremor was then measured 10 min after the administration of the oxotremorine. The total score for the group of mice was expressed as a percentage of the maximum possible score and the activity calculated by subtracting the percentage score from 100.

The activity was plotted against dose on log probit paper and the relative potency of benapryzine to benzhexol was estimated from the graphs.

Estimation of salivation in the mouse

The time of maximum activity as determined by the mydriatic response of benzhexol and benapryzine in mice occurs approximately 10 min after the subcutaneous injection and 20 min after oral administration. Benapryzine and benzhexol were administered orally and subcutaneously to groups of 12 male mice (19–22 g). Ten minutes prior to the period of maximum activity all the mice were lightly anaesthetized with 1·0 g/kg ethyl carbamate subcutaneously, and at the peak time the mice received 2 mg/kg pilocarpine nitrate subcutaneously, in order to induce a good flow of saliva.

The mice were placed in the prone position on a sloping (30°) sheet of glass which was covered with chromatographic paper which had been soaked in a 2% solution of sodium starch glycolate, the paper being ruled in 3 cm squares. The mice were advanced to a new square every 5 min for a total of 30 minutes. The areas of saliva were allowed to dry and the paper was then sprayed with iodine solution. The areas where the salivary amylase had hydrolyzed the starch showed up white against the starch iodine coloured background and these areas which represented the amount of salivation were measured with an Albrit planimeter. Six areas in arbitrary units for each mouse were summed and the mean values used to calculate the percentage inhibition of salivary flow as compared to the control values.

Extrapyramidal effects

Groups of 5 male rats (Charles River Sprague-Dawley 200–250 g) were dosed with perphenazine 5 mg/kg i.p. and observed for the degree of catalepsy which developed. This was assessed by the method of Morpurgo (1962), in which each front paw is placed in turn on a small wooden block 2·5 cm high, then on a larger block 10 cm high. If the rat kept its paw on the block for longer than 20 s, then half a point was given for each front paw remaining on the small block and one point for each paw remaining on the large block. This gave a possible total score of 3 for each rat with a total score of 15 when full catalepsy had developed in the group. Benapryzine and benzhexol were administered 50 mg/kg orally 2 h after the perphenazine and the degree of catalepsy was observed for a further 2 h at 15 min intervals.

Studies in man

Estimation of salivation in man

The effect of benapryzine (mean total dose 60 mg/day) on salivation was compared with benzhexol (mean dose 12 mg/day) in six patients with Parkinson's disease. The patients' subjective complaint of a dry mouth (graded 0–4, in increasing severity) was recorded, and the amount of wetting of a piece of blotting paper of standard size held between the lips was measured in mm/minute. Each drug was taken for a week, and observations were made usually on alternate days, so there were 16 recordings while taking benapryzine and 13 recordings while taking benzhexol.

Central anticholinergic activity in man

Duvoisin (1967) has shown that intravenous injections of anticholinesterases which enter the brain (e.g. physostigmine) cause a profound increase of all the manifestations of Parkinson's disease; tremor, rigidity, and akinesia all become worse. This effect is abolished by central antiacetylcholine drugs (e.g. scopolamine or benztropine methanesulphonate), but not by antiacetylcholine drugs which do not penetrate the brain (e.g. methylscopolamine); nor is this effect reproduced by anticholinesterases which do enter the brain (e.g. edrophonium). A drug with effective central antiacetylcholine action should abolish the effects of physostigmine on the Parkinsonian syndrome.

Physostigmine salicylate (1 mg) and methylscopolamine bromide (1 mg) (to prevent the peripheral effects of the anticholinesterase) were injected i.v. into two patients with Parkinson's disease who volunteered for the experiment. A first injection was given when the patient had been taking a placebo medication for 48 h; the injection was repeated after the patient had been taking benapryzine for a week, in a dose of 10 mg, three times a day in one case, and 30 mg, three times a day in the other.

Effect of benapryzine on Parkinson's disease

Six patients (aged 54–76 years; four male, two female) with idiopathic paralysis agitans volunteered for the investigation which was designed to compare the effects of benapryzine with those of a placebo and benzhexol. All drugs were made up in

identical capsules and were given thrice daily before food. The patients were admitted to hospital for the study. All previous drugs were stopped on admission and placebo was substituted. After 48 h on placebo, benapryzine (10 mg, three times a day) or benzhexol (2 mg, three times a day) was given; the dose of each was increased daily by 10 mg or 2 mg respectively until side effects appeared. The maximum doses given were benapryzine 40 mg, three times a day and benzhexol 10 mg, three times a day. After a week's treatment, placebo was substituted for the active drugs for 48–96 hours. Then each patient was switched to the other active drug for a further week. Each patient thus took benapryzine for a week, benzhexol for a week, and placebo on two occasions for at least two days each time. Four of the six patients took benapryzine followed by benzhexol; two patients took benzhexol before benapryzine.

One observer, who was unaware of which drug the patient was taking, assessed each patient at least twice weekly. Rigidity (clinical grading 0–5 scale), tremor (5 min recording by the technique of Cowell, Marsden & Owen, 1965), and akinesia (assessed by repetitive grip strength and speed measured with a bulb ergometer after England & Schwab, 1959) were measured separately and a battery of tests was timed individually (walking ten yards with a turn, stand up and sit down, take off and replace a pair of shoes and socks, take off and replace a coat, do up buttons, open and close each fist ten times). Samples of handwriting and drawing were also obtained.

Observations were recorded on 16 occasions when patients were taking benapryzine and on 13 occasions when taking benzhexol. Results of each observation are expressed as a percentage improvement over the mean score for the two observations made on each patient when taking placebo. Throughout the study neither the patient nor the observer knew which medication was being administered.

Toxicity studies on benapryzine

The following investigations were carried out on each patient, first while taking placebo, then while receiving the maximum dose of benapryzine: plasma electrolytes and urea, cholesterol, uric acid, bilirubin, alkaline phosphatase, serum glutamic oxaloacetate transaminase, and fasting glucose; haemoglobin, white count, platelet count, blood film, erythrocyte sedimentation rate, urinalysis (cell, protein, glucose) and electrocardiograph.

Drugs. The following drugs were used: benzhexol and benapryzine (made by Beecham Research Laboratories), oxtremorine, ethyl carbamate, acetylcholine chloride, pilocarpine nitrate, physostigmine salicylate, methylscopolamine bromide and benztropine methaneosulphate.

Results

Studies in animals

In vitro *activity in the guinea-pig ileum*

The pA2 for benapryzine on the guinea-pig ileum against acetylcholine was found to be 6·55 and for benzhexol 9·02. The potency of benapryzine compared to benzhexol was 0·0027, with 95% limits of 0·0047 and 0·0017.

Time course of mydriatic activity

The results for the time course of mydriatic activity are shown in Figure 1. The graphs represent the mean mydriatic response of five animals but for the purposes of clarity the limits have been omitted. The mydriatic activity gives an assessment of peripheral anticholinergic activity and the figure shows that in addition to producing a much greater response, benzhexol has a much longer duration of action than benapryzine

Mydriatic activity in the mouse

The results of the direct assay of potency for mydriatic activity 20 min after subcutaneous administration are shown in Figure 2. Owing to the fact that the

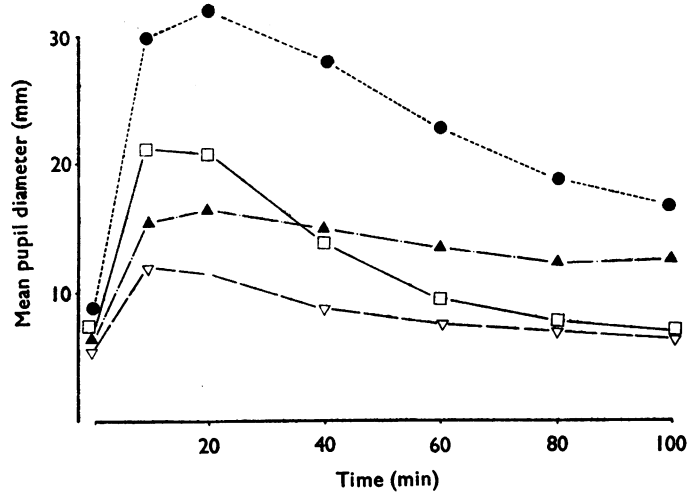

FIG. 1. Time course of the mean mydriatic activity in mice, 5 per group, following subcutaneous administration of benapryzine and benzhexol. Benapryzine, 20 mg/kg □——□; 10 mg/kg ▽——▽. Benzhexol, 0·8 mg/kg ●- - - -●; 0·4 mg/kg ▲—·—·▲.

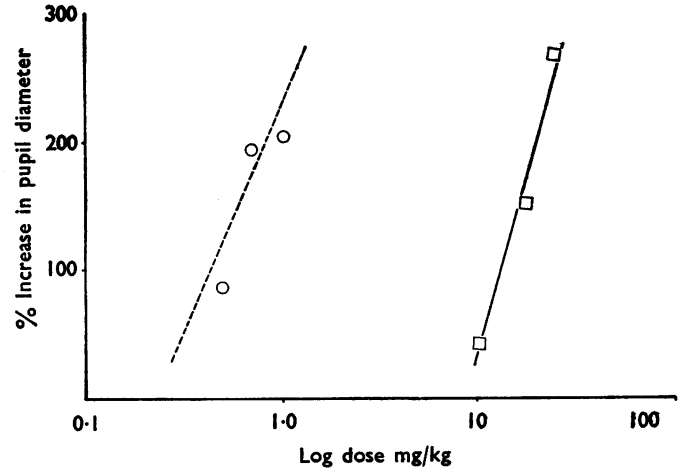

FIG. 2. Dose response lines for the mydriatic activity of benapryzine (□——□) and benzhexol (○- - -○) in mice measured 20 min after subcutaneous administration. Each point represents the mean response of five animals.

ratio of the high dose to the intermediate dose and the ratio of the intermediate dose to the low dose were not the same, the results were analysed by the method described by Brownlee (1965). The relative activity of benapryzine to benzhexol was found to be 0·038 with 95% confidence limits 0·031–0·046. Benapryzine was thus found to be thirty times less active than benzhexol in peripheral anticholinergic activity.

Anti-tremor activity

The effect of benapryzine and benzhexol in reducing the tremor induced by the intraperitoneal injection of oxotremorine is shown in Figure 3. The results were recorded as semi-quantal responses and the potency estimates were calculated by the method described by Gurland, Lee & Dahm (1960). The relative potency of benapryzine in relation to benzhexol is 1·1 (95% confidence limits 1·96–0·71) after subcutaneous injection, and 0·73 (95% confidence limits 1·94–0·51) after oral administration.

Antisalivary action

The activity of benapryzine and benzhexol in antagonizing pilocarpine-induced salivation is shown in Figure 4.

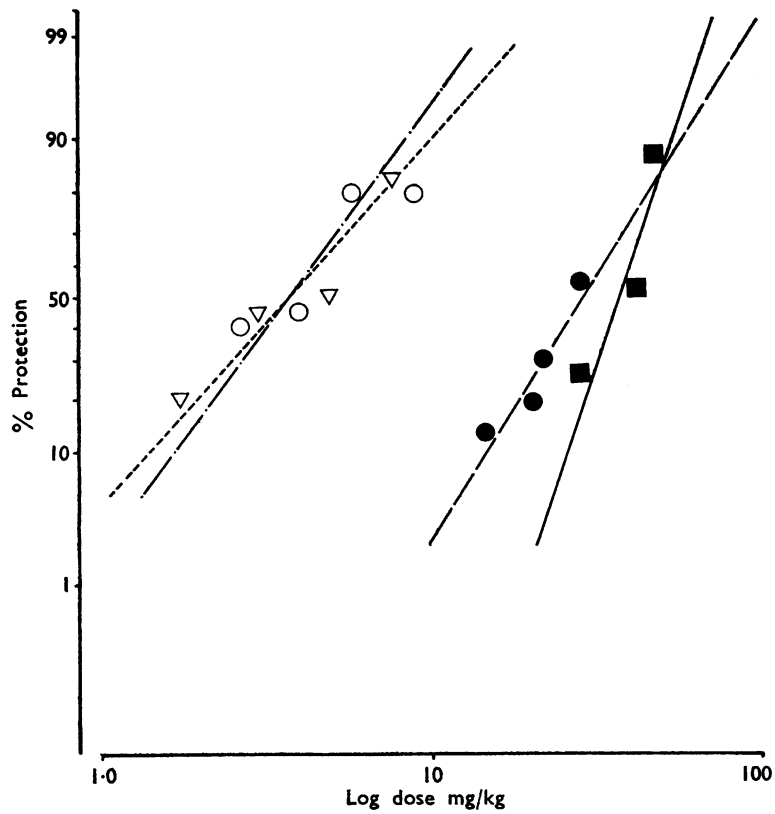

FIG. 3. Dose response lines for the anti-oxotremorine (2 mg/kg i.p.) activity in mice of benapryzine and benzhexol following oral and subcutaneous administration. Each point represents the mean response of 10 animals. Effect of benapryzine (△– – –△) and of benzhexol (○—·—·○) 20 min after s.c. injection. Effect of benapryzine ■——■ and of benzhexol (●– – –●) 40 min after oral administration.

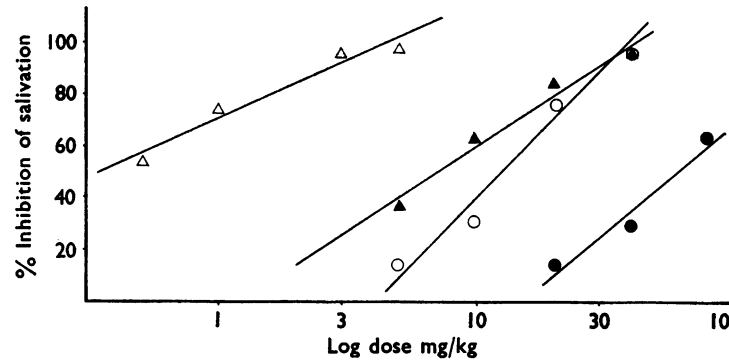

FIG. 4. Dose response lines for the antagonistic activity of benapryzine and benzhexol against pilocarpine-induced salivation in mice. Each point is the mean response of 12 mice. The drugs were given subcutaneously and orally, and 10 min prior to maximal activity the mice were sedated with 1·0 g/kg ethyl carbonate. At peak action 2 mg/kg pilocarpine nitrate was given subcutaneously. The effect on salivary flow was measured as described in the text. Benapryzine s.c.—○——○; orally—●——●. Benzhexol s.c.— △——△; orally—▲——▲.

For the oral comparison, the estimate of potency was calculated by the method of Finney (1952) and the ratio of the activity of benapryzine to benzhexol was found to be 0·13 (95% confidence limits 0·18 to 0·096).

Parallel lines could not be fitted to the data obtained from the subcutaneous experiment and an appropriate analysis could not be carried out. An approximate estimate of the potency ratio between benapryzine and benzhexol of 0·056 was found by taking the difference between the logs of the doses that gave 50% inhibition as estimated from the individual regression lines for both compounds.

Extrapyramidal effect

Benapryzine antagonized the catalepsy induced in the rat by perphenazine at doses of 50 mg/kg orally. The response and duration of activity did not differ significantly from animals given the same oral dose of benzhexol. The animals began to show signs of catalepsy returning about 1·5 h after administration.

Studies in man

Antisalivary action

Five patients complained repeatedly of a dry mouth while taking benzhexol but only one while taking benapryzine. There was no difference in the measured rate of salivation whether patients took benapryzine or placebo. Benzhexol decreased salivation when compared with benapryzine in the same patients ($t=4·05$; $P<0·01$).

Central anticholinergic activity

Within five minutes of the intravenous injection of physostigmine, both patients became very much more incapacitated by their Parkinson's disease (a prior injection of saline had not had this effect). Subject HH. became so tremulous that he could no longer sign his name, and so akinetic and rigid that he could no longer walk unaided; he could only just rise from a chair. Subject AB. was affected less seriously, but tremor increased tenfold and the grip strength and speed of grip (measured by bulb ergometer) deteriorated to about 75% of control values.

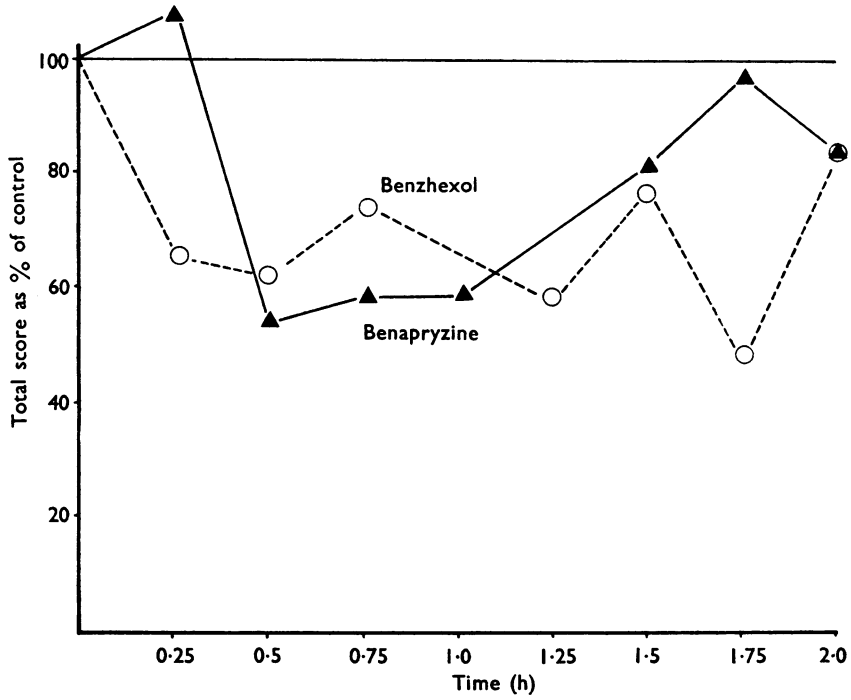

FIG. 5. Inhibition of catalepsy in rats produced by perphenazine (5 mg/kg i.p.). Benapryzine (▲) and benzhexol (○) administered orally (50 mg/kg) 2 h after perphenazine. The severity of the catalepsy was assessed by a scoring system and summed for each group of 5 rats and expressed as a percentage of the untreated control group.

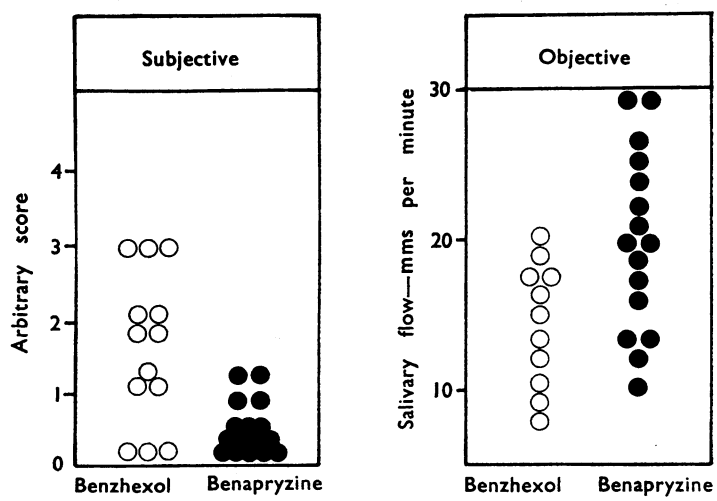

FIG. 6. Subjective and objective estimates of salivation obtained in six patients following benzhexol and benapryzine. The subjective complaint of a dry mouth was evaluated on a 0–4 scale, 0 indicating no complaint. The objective measure is of degree of wetting (in millimetres per minute) of a standard strip of blotting paper between the lips.

Subsequently benztropine methaneosulphate (2 mg) was injected intravenously, and both patients returned to their previous state within 20 minutes.

When the injection of physostigmine was repeated one week later after taking benapryzine, neither patient became worse; nor did they believe they had undergone the same procedure. Benapryzine thus abolished the central effects of physostigmine.

Anti-Parkinsonian activity

Figures of per cent improvements over placebo for rigidity, ergographic performance (a measure of grip strength, grip speed, and fatigue of grip) and the average of the results of the timing tests are shown in Table 1 and Figure 7.

Both benapryzine and benzhexol produced a small but consistent improvement in all the parameters of the Parkinsonian syndrome; there was no significant difference between the slightly better results obtained with benzhexol and those with benapryzine. Only three patients had significant tremor, which got worse with benapryzine in two patients and better in one.

TABLE 1. *Mean % improvement in rigidity, timing tests and ergographic performance in six patients receiving benapryzine* (30 *to* 120 *mg daily:* 16 *observations*) *and benzhexol* (6 *to* 30 *mg daily:* 13 *observations*)

	Benapryzine		Benzhexol	
	Mean % Improvement	Range	Mean % Improvement	Range
Rigidity	24	(−6 to 85)	30	(−5 to 65)
Ergographic timing tests	24	(−5 to 53)	34	(12 to 76)
Ergographic performance	15	(−9 to 47)	26	(0 to 48)
Mean:	21		30	

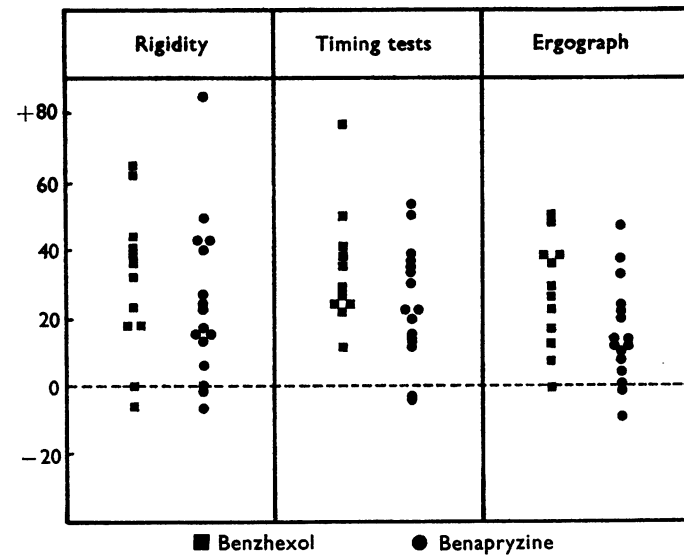

FIG. 7. Percentage improvement over placebo scores obtained in individual observations of rigidity, timing tests and ergographic performances in 6 patients after benzhexol (■) and benapryzine (●).

There was no apparent increase in benefit from high doses of benapryzine (60–120 mg daily) compared with that obtained from low doses (30–45 mg daily).

Side effects

Side effects of benapryzine were rare. Two patients noticed drowsiness (on 90 mg and 75 mg daily respectively), and one patient reported a dry mouth (on 60 mg daily). No psychotoxic reactions occurred.

Two patients taking benzhexol (in doses of 15 mg and 12 mg daily respectively) developed confusion, disorientation, and hallucinations. Neither patient had such an experience while previously taking benapryzine (in doses of 120 mg and 90 mg daily respectively). These psychotoxic reactions to benzhexol disappeared when the drug was withdrawn.

One patient developed marked postural syncope with measurable postural hypotension while taking benzhexol (24 mg daily); this disappeared when the drug was withdrawn and did not recur when benapryzine was substituted.

Five of the six patients complained of a dry mouth while taking benzhexol, but only one patient noticed this while taking benapryzine. One patient, however, preferred the dry mouth produced by benzhexol, for when he took benapryzine he developed profuse sialorrhea.

Toxicity studies

None of the blood or urine constituents was altered by benapryzine, nor was the E.C.G. affected.

Discussion

The anti-oxotremorine action in mice of benzhexol and benapryzine were equivalent after both oral and subcutaneous administration whereas the peripheral anti-acetylcholine action of benzhexol as exemplified by the mydriatic action and the effect on salivary flow was many times greater than the corresponding effect caused by benapryzine.

The tremor induced by tremorine and oxotremorine in animals is believed to be mediated through central cholinergic pathways (Everett *et al.*, 1956; Cox & Potkonjac, 1969) and in man the symptoms of Parkinson's disease exacerbated by anticholinesterases are mediated by a central mechanism (Duvoisin, 1967). Initial pharmacological investigations in man have demonstrated that benapryzine and benzhexol antagonize the action of physostigmine in Parkinsonian subjects, thus indicating that both drugs act centrally in man. On the other hand in man, as in animals the peripheral anticholinergic actions of benapryzine are virtually absent. The reason for this can partly be accounted for by the fact that it is rapidly metabolized peripherally, only 7% being recovered in the urine in 24 hours. Eight metabolites, accounting for the remainder of the drug administered, have been identified, the principal one being ethylpropylaminoethanol. Of the radioactivity recovered about 70% appeared in the faeces and the remainder in the urine following subcutaneous administration (Jeffery, Brown & Langley, 1971).

Following oral administration to rats the concentration of benapryzine in the blood is constant, in the range 2·4–5·2 ng/ml over a period of 4 h, at any given time.

These values represent only 3–8% of the total amount of radioactivity in the blood (unpublished results). The intrinsic *in vitro* antiacetylcholine activity of the compound is quite low, however, and it could be that the enhanced central action is due to the fact that sufficient compound penetrates brain neurones where it can exert a pharmacological action and where it is metabolized at a slower rate.

Benapryzine can antagonize in rats the catalepsy induced by a major tranquillizer perphenazine. Its effect and duration of action is similar to that of benzhexol and it could therefore be of value in controlling iatrogenic extrapyramidal symptoms induced by tranquillizers.

Benapryzine in addition to its anti-acetylcholine action antagonizes both maximal electroshock and metrazol-induced convulsions in mice. This feature is not generally shown by anti-acetylcholine agents but is seen with orphenadrine (unpublished observations).

Current clinical trials with benapryzine have confirmed its usefulness in controlling the symptoms of Parkinson's disease and while evidence is accumulating that the role of a central cholinergic system in Parkinsonism is secondary to the primary role of a dopaminergic system, future therapy will be in the control of the disease by a balance of drugs acting to regulate both systems.

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